

This further demonstrated the pivotal role of the Th1/Th2 balance in regulation of T cell mediated autoimmunity (38).

IGIF is a recently described cytokine (25) that shares structural features with the interleukin-1 (IL-1) family of proteins (26). Activation of IGIF is mediated by interleukin-1 beta converting enzyme (ICE) (27, 28). Like IL-12, IGIF is a potent inducer of IFN- $\gamma$  from Th1 and NK cells, and acts on Th1 cells together with IL-12 in a synergistic manner (25, 29-32). IGIF actually has more potent IFN- $\gamma$  inducing capabilities than IL-12 and apparently utilizes a distinct signal transduction pathway for its elicitation (25, 31, 32, 51). Little is known about the role of IGIF in T cell mediated autoimmune disease. A recent study used RT PCR to demonstrate that the active stage of autoimmune diabetes in NOD mice is associated with the expression of IGIF (52).

As further detailed in the Examples section below, while conceiving and reducing the present invention to practice, specific oligonucleotide primers were used to identify and isolate interferon gamma inducing factor (IGIF) from the brain of rats with developing experimental autoimmune encephalomyelitis (EAE), a T cell mediated autoimmune disease of the central nervous system (CNS) that serves as a model for multiple sclerosis (MS).

IGIF was highly transcribed in the brain at the onset and during the course of active EAE. PCR products encoding rat IGIF were used to generate the recombinant protein which was used to induce anti-IGIF neutralizing antibodies. These antibodies significantly reduced the production of interferon gamma (IFN- $\gamma$ ) by primed T cells proliferating in response to their target myelin basic protein (MBP) epitope and by Con A activated T cells from naive donors.

When administered to rats during the development of either active or transferred EAE, these antibodies significantly blocked the development of disease.

Splenic T cells from protected rats were cultured with the encephalitogenic MBP epitope and evaluated for production of IL-4 and IFN- $\gamma$ . These cells, which proliferated, exhibited a profound increase in IL-4 production, accompanied by a significant decrease in IFN- $\gamma$  and TNF- $\alpha$  production.

An elevated expression of IGIF at the time when the secondary influx of autoimmune cells is apparent at the site of inflammation in the EAE brain (38, 39, 46, 53) is demonstrated herein for the first time. So are

neutralizing antibodies, which were generated against IGIF cloned from this site of inflammation, to block the disease by altering the *in vivo* Th1/Th2 balance in favor of Th2 selection. This alteration included a marked reduction in the production of IFN- $\gamma$ , and, most importantly, TNF- $\alpha$ , a proinflammatory cytokine that plays a critical role in T cell mediated autoimmunity (44, 47-50).

An interesting observation is that both the inhibitory effect of IGIF neutralizing antibodies and the augmentation by IGIF of IFN- $\gamma$  production are more profound on activated T cells from a naive donor than on primed T cells responding to their target epitope.

The direct role of IFN- $\gamma$  in EAE is enigmatic. Grewal et al. have used a CD40L-deficient mice that carry a transgenic T cell receptor specific for MBP to demonstrate that EAE induction is IFN- $\gamma$  dependent (54). On the other hand not only were mice lacking IFN- $\gamma$  susceptible to induction of active EAE (55) but also antibodies to IFN- $\gamma$  were found capable of enhancing this disease (56, 57). A recent study has demonstrated that IL-12 is directly involved in the generation of autoreactive Th1-cells that induce EAE, both in the presence and the absence of IFN- $\gamma$  (58). However, it could well be that alteration the Th1/Th2 balance towards IL-4 secreting Th2 cells confers EAE resistance not because it leads to a reduced production of IFN- $\gamma$ , but rather because it results in a reduced production of TNF- $\alpha$  accompanied by a marked increase in IL-4 production.

It has recently been suggested that IGIF primarily effects IFN- $\gamma$  production by Th1 but not Th2 cells (29). It is possible that immunization with p68-86/CFA induces a substantial selection of antigen specific Th2 cells, albeit not enough to inhibit the subsequent development of a Th1 mediated autoimmune disease. Hence, as shown herein, *in vivo* administration of anti IGIF neutralizing antibodies notably shift the Th1/Th2 balance in antigen specific proliferating T cells towards Th2 response.

Thus, the present invention teaches the use of an anti interferon gamma inducing factor antibody in the treatment of multiple sclerosis. This use can be effected in a variety of ways and applications, some of which are further described and exemplified hereinbelow.

According to one aspect of the present invention there is provided an antibody which comprises an immunoglobulin capable of binding interferon gamma inducing factor (IGIF, IL-18).

As used herein in the specification and in the claims section below, the terms "antibody" and "immunoglobulin", which are interchangeably used, refer to any of several classes of structurally related proteins that function as part of the immune response of an animal, which proteins include IgG, IgD, IgE, IgA, IgM and related proteins. These terms further relate to chimeric immunoglobulins which are the expression products of fused genes derived from different species. These terms further relate to immunologically active derivatives of the above proteins, including, but not limited to, an F(ab')<sub>2</sub> fragment, an Fab fragment, an Fv fragment, a heavy chain, a light chain, an unassociated mixture of a heavy chain and a light chain, a heterodimer consisting of a heavy chain and a light chain, a catalytic domain of a heavy chain, a catalytic domain of a light chain, a variable fragment of a light chain, a variable fragment of a heavy chain, and a single chain variant of the antibody. Under normal physiological conditions antibodies are found in plasma and other body fluids and in the membrane of certain cells and are produced by lymphocytes of the type denoted B cells or their functional equivalent. Antibodies of the IgG class are made up of four polypeptide chains linked together by disulfide bonds. The four chains of intact IgG molecules are two identical heavy chains referred to as H-chains and two identical light chains referred to as L-chains. The immunoglobulin or antibody according to the present invention could also be a "humanized" antibody, in which, for example animal (say murine) variable regions are fused to human constant regions, or in which murine complementarity-determining regions are grafted onto a human antibody structure (Wilder, R.B. et al., J. Clin. Oncol., 14:1383-1400, 1996). Unlike, for example, animal derived antibodies, "humanized" antibodies often do not undergo an undesirable reaction with the immune system of the subject. The terms "sFv" and "single chain antigen binding protein" refer to a type of a fragment of an immunoglobulin, an example of which is sFv CC49 (Larson, S.M. et al., Cancer, 80:2458-68, 1997). As used herein, the term "humanized antibodies" also reads on antibodies produced by non-human cells or organisms genetically modified to include nucleic acid sequences encoding a functional portion of the human immune system, wherein the resulting antibodies are substantially identical to human antibodies in that they are encoded by human derived genes. However, the term "antibody", as used herein, further relates to soluble portions of receptors capable of specifically binding their respective protein ligands, which, in that respect, function like immunoglobulins.